

Autochthonous dengue fever in Croatia, August–September 2010

I Gjenero-Margan (epidemiologija@hzjz.hr)¹, B Aleraj¹, D Krajcar², V Lesnikar², A Klobučar², I Pem-Novosel¹, S Kurečić-Filipović¹, S Komparak³, R Martić³, S Đuričić⁴, L Betica-Radić⁴, J Okmadžić⁵, T Vilibić-Čavlek⁴, A Babić-Erceg⁴, B Turković⁴, T Avšič-Županc⁶, I Radić¹, M Ljubić⁷, K Šarac¹, N Benić², G Mlinarić-Galinović¹

1. Croatian National Institute of Public Health, Zagreb, Croatia
2. Public Health Institute of the City of Zagreb 'Dr. A. Štampar', Zagreb, Croatia
3. Dubrovnik–Neretva County Public Health Institute, field unit Korčula/Pelješac, Korčula, Croatia
4. Dubrovnik County Hospital, Infectology ward, Dubrovnik, Croatia
5. Primary Health Care Unit Orebić, Orebić, Croatia
6. Institute for Microbiology and Immunology, Medical faculty, Ljubljana, Slovenia
7. Public Health Institute of Dubrovnik Neretva County, Epidemiology service, Dubrovnik, Croatia

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After information about a dengue case in Germany acquired in Croatia, health professionals and the public in Croatia were alerted to assess the situation and to enhance mosquito control, resulting in the diagnosis of a second case of autochthonous dengue fever in the same area and the detection of 15 persons with evidence of recent dengue infection. Mosquito control measures were introduced. The circumstances of dengue virus introduction to Croatia remain unresolved.

Introduction

The epidemiology service of the Croatian National Institute of Public Health (CNIPH) has registered six imported cases of dengue virus (DENV) infection since 2007. All except one were Croatian citizens who had spent time in areas with local transmission of this disease (Southeast Asia, South America) and had a mild clinical presentation of dengue fever. The sixth case occurred in 2007 in a tourist visiting Croatia who developed haemorrhagic fever and had previously travelled in South-east Asia [1,2]. Although a seroepidemiology study conducted in 1980 in a limited area of north-eastern Croatia in healthy young inhabitants proved the presence of antibodies to DENV type 2 (3,9%) and type 1 (2,1%) [3], no cases of dengue fever were registered by the health services.

Aedes albopictus was for the first time recorded in Croatia in 2004 in the area surrounding Zagreb [4]. Within two years, *Ae. albopictus* was found on the entire territory of the Adriatic coast from northern Istria to Dubrovnik in the south. According to routine monitoring of mosquitoes and published articles, *Ae. albopictus* is now permanently established in the coastal but not yet in the continental areas of Croatia [5].

Soon after reports on the first autochthonous DENV infection diagnosed in France in September 2010 [6], the Epidemiology Service of the CNIPH was notified on 30 September by the Robert Koch Institute (RKI) in Germany of a German citizen, who fell ill with symptoms of dengue fever immediately after returning to Germany from a 15-day stay on the Pelješac peninsula in Croatia. Virological investigation revealed the presence of DENV-specific IgM, a rise in DENV-specific IgG and the presence of NENV NS1 antigen in the patient's blood [7]. As this was the first case of dengue fever probably acquired in Croatia, an epidemiological investigation was conducted and outbreak control measures implemented. We present here the first results of the epidemiological investigation.

Active case finding

According to information received by the RKI, the German citizen travelled from Germany via Austria and Slovenia along the Croatian coast in August 2010 and stayed on the Pelješac peninsula and the island of Korčula for 15 days. The disease onset was on the day after he returned to Germany. The information received from RKI on the first autochthonous case of dengue fever was sent to the World Health Organization (WHO) via the International Health Regulations (IHR) information network [8] by the national IHR focal point (the epidemiology service of the CNIPH) and disseminated to the Croatian and international public via the media in order to increase local and international awareness.

The epidemiological investigation started in Pelješac and Korčula, and will be gradually expanded to the entire Croatian littoral. The CNIPH released a circular letter informing all epidemiology services and hospital infectology clinics in the country to consider the possibility of dengue fever in clinically compatible cases including those with no history of travelling

abroad. The circulatory letter contained the entire range of clinical manifestations of dengue fever, and for the purposes of the epidemiological investigation, a question on diseases accompanied with fever occurring in the village where the German tourist had stayed. At the time of the first epidemiological investigation among local physicians in the beginning of October 2010, there were no such acute diseases in the area. In the following weeks, a number of clinically suspect cases were reported and serum samples were sent to the CNIPH, but tested negative for dengue virus (Anti-Dengue virus Elisa IgM/IgG, Euroimmun, Germany).

On 22 October 2010, a possible case of dengue fever was reported in a resident of the same village where the German patient had stayed. The Croatian patient, a woman in her fifties, who had not travelled outside her place of residence, developed symptoms compatible with dengue fever on 17 October, including temperature up to 39°C, skin rash, chill, headache and joint and muscle pain, and was admitted to the infectology ward at Dubrovnik hospital on day 6 after onset of disease.

Laboratory diagnosis

A serum sample was taken from the Croatian patient on admission to hospital and sent to the National Reference Laboratory for Arboviral Infections at the Virology department of the CNIPH in Zagreb. Virological analysis by ELISA (Anti-Dengue virus ELISA IgM/IgG Euroimmun, Germany) detected DENV-specific IgM (ratio 1.9). The sample was negative for DENV-specific IgG (10 relative units (RU)/ml), West Nile virus (WNV) IgM and IgG (Anti-West Nile virus ELISA IgM/IgG, Euroimmun, Germany), and chikungunya virus IgM and IgG. (Anti-Chikungunya virus ELISA, IgM/IgG, Euroimmun, Germany). The patient had not been vaccinated against yellow fever or tick-borne encephalitis (TBE). The village of residence as well as whole southern Croatia is not endemic for TBE [9]. Considering the current epidemiological situation in Croatia these laboratory results pointed to a diagnosis of dengue fever [10].

The patient's first serum was also sent to the regional reference laboratory in Ljubljana (Slovenia) where an RT-PCR was negative for dengue virus [11]. The second (paired) serum was taken on day 19 after onset of illness when the patient was already discharged and recovered, and the sample was analysed at CNIPH with the following results: DENV: IgM-positive (ratio 4.9) and IgG-positive (110 RU/ml); WNV: IgM-positive (ratio 1.2) and IgG-negative; and chikungunya virus: IgM- and IgG-negative. These results confirmed this case as the second autochthonous case of dengue fever in Croatia.

Analysis of serum samples collected in the area

We collected 14 blood samples from healthy inhabitants living near the case's place of residence. The samples were analysed by ELISA for the presence of DENV and WNV IgM/IgG antibodies. Nine of those were

found positive for DENV infection (IgG) and seven had positive or borderline results for DENV-specific IgM (Table 1).

A further 112 sera collected from anonymous patients who had sought medical care from various reasons during October 2010 were available at the laboratory of the local health centre. These sera were tested at

TABLE 1

Distribution of antibodies to dengue virus in nine persons from a pool of 14 neighbours of the autochthonous case from Pelješac, Croatia, October 2010

| Examinee number | DENV IgM (ratio) ^a | DENV IgG (RU/ml) ^b |
|-----------------|-------------------------------|-------------------------------|
| 1 | + (2.4) | + (155) |
| 2 | +/- (1.04) | + (126) |
| 3 | + (2.2) | + (98) |
| 4 | + (1.2) | + (140) |
| 5 | - (0.3) | + (170) |
| 6 | - (0.5) | + (155) |
| 7 | + (2.3) | + (138) |
| 8 | + (2.4) | + (94) |
| 9 | + (2.6) | + (170) |

DENV: dengue virus.

^a <0.8 negative (-), 0.8-1.1 borderline (+/-), ≥1.1 positive (+). Results are expressed as ratio according to the manufacturer's specifications.

^b <16 negative (-), 16-22 borderline (+/-), ≥22 positive (+). Results are expressed in RU/ml according to the manufacturer's specifications.

TABLE 2

Distribution of antibodies to dengue virus in six anonymous serum samples, Croatia, October 2010

| Examinee number | 1 | 2 | 3 | 4 | 5 | 6 |
|-------------------------------|-----------|-----------|------------|---------|----------|---------|
| DENV IgM (ratio) ^a | +/- (0.9) | +/- (0.8) | +/- (1.08) | + (4.6) | + (2.2) | - |
| DENV IgG (RU/ml) ^b | + (72) | + (46) | + (40) | + (46) | +/- (12) | + (153) |

DENV: dengue virus.

^a <0.8 negative (-), 0.8-1.1 borderline (+/-), ≥1.1 positive (+). Results are expressed as ratio according to the manufacturer's specifications.

^b <16 negative (-), 16-22 borderline (+/-), ≥22 positive (+). Results are expressed in RU/ml according to the manufacturer's specifications.

TABLE 3

Adult mosquitoes caught in Podobuče, Orebić and Korčula, Croatia, October 2010

| Species | Number |
|----------------------------|-----------|
| <i>Aedes albopictus</i> | 49 |
| <i>Ochlerotatus mariae</i> | 4 |
| <i>Ochlerotatus sp.</i> | 2 |
| <i>Culex pipiens</i> | 5 |
| <i>Culiseta annulata</i> | 1 |
| Total | 61 |

CNIPH for the presence of DENV and WNV antibodies. Ethics approval was not required and informed consent was not sought. The work was carried out under the Communicable Disease Protection and Control Act which provides statutory support for investigations conducted for the purpose of communicable disease control.

Of those 112 samples, six were positive for DENV-specific antibodies. In all six positive sera, DENV-specific IgG was found (one sample with a borderline value). DENV-specific IgM were found in five sera: clearly positive in two and borderline in three (Table 2). All 112 sera were negative for WNV IgM and IgG.

Entomological investigation

During the field investigation the presence of mosquitoes was noticed, despite mandatory disinsection implemented on the entire Croatian territory. Mosquitoes were caught in the place of probable transmission and also on the island of Korčula in October 2011 with the aim of identifying the mosquito species present and determining whether they were carrying DENV. Two days after the mosquito had been caught, adulticidal and larvicidal disinsection was conducted at the village where the German patient had stayed.

The 61 caught mosquitoes were identified by an entomologist (Table 3). The species *Ae. albopictus* dominated (49 of 61).

Virological investigation was conducted for 44 *Ae. albopictus* adults in eight pools containing between five and seven mosquitoes. All eight pools tested negative for DENV in the RT-PCR conducted at the WHO Regional Reference Laboratory for Arboviruses at the Institute for Microbiology and Immunology in Ljubljana, Slovenia [11].

Discussion

After France, Croatia is the second country in Europe in which autochthonous transmission of dengue infection has been shown, which had not been recorded in Europe since the epidemic in Greece in 1925 to 1928 [12-15]. According to data of the communicable disease epidemiology service of the CNIPH, registered imported cases of dengue are not frequent (six cases in three years). Although until recently dengue fever was not a notifiable disease in Croatia, it is unlikely that the epidemiology service network which collaborates with the laboratories that conduct the diagnosis would have missed the occurrence of a confirmed case of imported dengue fever.

The assumption that the German tourist acquired dengue fever in the region of the Pelješac peninsula was confirmed by the identification of a second case of dengue fever in a local citizen who had not travelled outside the area. Although the antigen was not confirmed by RT-PCR in the acute serum of the patient, taken six days after illness onset, the presence of specific IgM

antibodies (IgG was negative) pointed to acute infection. This was confirmed in a sample taken on day 19 of the illness when IgM and IgG antibodies were found.

Nine of the 14 samples taken from the Croatian patient's neighbours, none of whom had travelled outside Croatia, were IgG-positive, and we assume that these were relatively recent infections because seven of them were also IgM-positive. Some of them reported having an influenza-like disease in August and early September. Moreover, DENV-specific antibodies were found in 5.4% of the anonymous serum samples collected in October 2010 by the laboratory that covers the area of Pelješac and Korčula. Five of the six DENV-positive sera in this panel showed borderline or positive values of IgM antibodies against DENV. Based on the available serological and epidemiological data we therefore assume that a cluster of acute DENV infection occurred in the area, most probably during August and September 2010.

Each cluster of infectious diseases is reported using the national communicable diseases early warning system. During summer 2010 there were no such reports from Pelješac. Only four of the DENV-positive villagers contacted health services for febrile illness in August and September and were not recognised as an outbreak. At that time of year, there is an increased circulation of enteroviruses which can manifest with similar symptoms, but we believe that the main reason why dengue fever was not suspected in these patients is the fact that this illness had not been registered in Croatia or Europe so far and is therefore not considered in persons who have no travel history to endemic areas. Although the health services had been alerted of the possibility of new diseases transmitted by *Ae. albopictus*, particularly following the chikungunya fever outbreak in Italy in 2007 [16], it was only after our circulatory letter in October that dengue virus infection in local inhabitants was suspected by general practitioners and subsequently confirmed in the second autochthonous Croatian case described in this paper. There may also have been other dengue virus infections with an inapparent or mild course. We believe that other tourists staying in the area may have returned to their home countries with dengue fever, but there have been no reports of exported cases other than the German case.

It is likely that the dengue virus was imported into this community during the summer months of 2010. Regarding the manner of importation, we can only speculate. It could have been through infected travellers arriving from endemic areas in whom the infection was not recognised. Bearing in mind that *Ae. albopictus* species have spread along the Adriatic coast, also in the region of Pelješac and Korčula mainly through transport by sea [5], importation of infected mosquitoes in the same manner cannot be excluded. Since the role of transovarial transmission in mosquitoes is questionable [17,18], it is not likely that importation

happened through infected eggs or larvae (e.g. in used car tires).

Croatia will continue to alert health practitioners to the presence of this disease. Larvicidal and adulticidal mosquito control measures already applied in the affected area will be continued and expanded to the entire country to prevent further establishment of dengue fever or other diseases transmitted by the same vector, such as chikungunya fever [19,20]. Selection of blood donors in the country is in line with all internationally accepted criteria, in that any febrile illness in a potential donor presents a contraindication including the period of convalescence. This covers dengue virus infections well, as cases are only infective during the febrile phase and there is no chronic infection stage. A short prodromal period can pose a risk, but the regular delayed use of blood donations allows to investigate all donors who fall ill in the first two days after donation and to discard their blood if necessary. However, the possibility of transient asymptomatic dengue viraemia needs to be taken into account in practice if the disease were to become established.

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